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Inside the shell: body composition of free-ranging tortoises (*Testudo hermanni*)

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25 **Abstract**

26 Body condition indices (BCI – mass scaled by size) are widely used in ecological
27 studies. They presumably reflect variations of endogenous fat reserves in free-
28 ranging animals. In the field, however, accurately quantifying internal body reserves
29 is a difficult task. This is especially true in armoured animals where convenient clues
30 that may guide BCI assessment (e.g. visible subcutaneous fat deposits) remain
31 inaccessible. Alternatively, inclusive dissections may provide anatomical abacuses to
32 estimate body reserves in living individuals. Sacrificing animals for this purpose is
33 not acceptable. We opportunistically tested the ability of BCI to estimate body
34 reserves in 13 free-ranging Hermann's tortoises (Gmelin, 1789) dissected soon after
35 they died from natural causes. On average, BCI values were lower in dissected
36 tortoises relative to living individuals (N>10,000 measurements), but they remained
37 within the range of variation of the studied populations. Shell mass relative to body
38 mass was high and showed considerable inter-individual variation (33.5% to 52.3%).
39 Stomach and digestive tract content represented another important and variable part
40 of total body mass (4.4% to 14.5%). The contribution of fat bodies was negligible
41 (0.0% to 0.5%). Overall, in the studied tortoises, variations of body condition are
42 weakly determined by variations of fat stores. Other endogenous (e.g. muscles,
43 visceral tissues, liver) and “exogenous” (e.g. digestive tract content, clutch) elements
44 should be considered to better understand age and sex specific life-history trade-offs
45 faced by chelonians.

46

47

48 Key words: Body condition index; fat reserves; dissection; Chelonians

49

50 **1. Introduction**

51 Direct access to the internal elements that compose an animal, for example
52 reproductive organs, body reserves or digestive tract content, is essential to examine
53 a wide range of questions in ecology, evolutionary ecology and toxicology (Schmidt-
54 Nielsen, 1975; Sakai et al., 2000; Zera and Harshman, 2001; Polilov, 2015). But in
55 Vertebrates, most studies have focused on a single segment. Typically, diet has been
56 assessed by scrutinizing stomach contents while other organs were overlooked
57 (Evans and Lampo, 1996; Paltridge et al., 1997). Similarly, during inspection of
58 ovarian structures, liver or muscles were generally ignored (Sica et al., 2001; Pizzatto
59 et al., 2008; Gherissi et al., 2018). Whole-body composition based on dissection has
60 been examined in only a handful of studies (Sakai et al., 2000; Landgraf et al., 2006).

61 Thus, for the vast majority of living Vertebrates, we cannot quantify the respective
62 input of each main body components to overall body mass (e.g. relative mass of the
63 skin or locomotor muscles). This limits the possibility to address ecological issues
64 where body condition indices (BCI, mass scaled by size) are used to evaluate lipidic
65 reserves (Stephens et al., 2009). For example, calculation and interpretation of BCI
66 can be misleading since total body mass includes unknown shares of fat stores,
67 muscles, partly digested food, and ovulated follicles. In most cases, measuring all
68 main internal body components is not feasible in the field, even when using
69 sophisticated techniques (Speakman, 2001; Cojean et al., 2018; Hofmeyr et al., 2020).
70 Currently, only comprehensive dissections allow isolating and measuring organs
71 (mass, dimensions), extracting and weighing whole content of the stomach or of the
72 intestine, to exactly assess body reserves, etc., and thus to eventually obtain a
73 comprehensive picture of the elements that constitute an animal as a whole.

74 But performing inclusive dissections relies on the availability of dead animals and
75 on the willingness to devote a considerable amount of time to the partitioning and
76 measuring of internal elements (Sakai et al., 2000). Although sacrificing free ranging
77 animals should be avoided, accidentally or naturally dead animals can be collected
78 without raising major ethical concern. Picking and fully dissecting randomly
79 encountered cadavers is not in the culture of most field ecologists.

80 The lack of convenient technique to assess an individual's body composition
81 might prove particularly taxing when dealing with animals protected by an external
82 armour (e.g. shell, large scales, spines or very thick skin). First, the mass of external
83 protection may well be substantial (e.g. ~30% of total body mass in pangolins;
84 Wilson and Mittermeier 2011). Second, the mass of all internal components that do
85 not belong to the tissues of the individual *sensu stricto*, like digestive tract content
86 (e.g. faeces) or developing embryos for example, is inaccessible via palpation
87 (ultrasound and radiography enable the visualization of follicles and eggs, however
88 - Hofmeyr et al., 2020). Third, convenient clues to assess BCI, like visible fat
89 deposits, cannot be used (e.g. camel hump; Gherissi et al., 2018). Overall, the
90 relationship between body condition and actual endogenous body reserves is
91 obscured using mass/size relationships, since exogenous matter and reproductive
92 items also contribute to overall body mass. These complications are almost
93 systematically ignored in the ecological literature where body condition is
94 considered as a reliable estimator of fat reserves (Schulte-Hostedde et al., 2005; Peig
95 and Green, 2009). Yet, the paucity of information also implies that we cannot gauge
96 the severity and relevance of this issue.

97 In this study we dissected fresh, naturally dead tortoises in the course of capture-
98 mark-recaptures studies. This offered the opportunity to examine to what extent the
99 use of a classical BCI may have tarnished our ecological interpretations during
100 previous studies (Bonnet et al., 2001; Djordjević et al., 2011; Lecq et al., 2014). Finally,
101 we expected that a better knowledge of tortoise body composition may improve
102 further ecological studies. To the best of our knowledge, this study provides the first
103 precise data on the whole-body composition of terrestrial Chelonians, and provides
104 exemplary points of prudence when interpreting BCI.

105

106 **2. Material and methods**

107 *Study species and study sites*

108 The Hermann's tortoise (*Testudo hermanni*) can grow up to 25 cm, attain 50 years, and
109 displays delayed maturity and low fecundity (Bertolero et al., 2011). Estimates for

110 body size at maturity range between 13 cm and 15 cm of straight carapace length
111 (SCL); we classified individuals larger than 13 cm as adults (Willemsen and Hailey,
112 1999).

113 From 2008 until 2019, we studied the ecology and morphology of tortoises in two
114 dense populations (> 20 adults / ha) of *T. hermanni* in two nearby sites (~ 4.5 km
115 away from each other) of the Prespa Lake region in North Macedonia: Golem Grad
116 Island (GG, ~ 18 ha; N $40^{\circ}52'$; E $20^{\circ}59'$), and Konjsko village (KV, ~ 20 ha, N $40^{\circ}54'$; E
117 $20^{\circ}99'$) (Djordjević et al., 2011; Arsovski et al., 2018a; Golubović et al., 2013, 2017,
118 2018).

119

120 *Data collection*

121 Each individual was sexed when possible (small individuals cannot always be sexed
122 – Arsovski et al., 2018b; Golubović et al., 2018) and marked using a notch-code on
123 the marginal-scutes (Stubbs et al., 1984). Overall, we encountered tortoises more than
124 18,500 times in the field (2,798 captures plus 15,789 recaptures). Straight carapace
125 length (SCL) and body mass (BM) were recorded, and individuals were rapidly
126 released at the exact place of capture. To limit unnecessary handling during frequent
127 recaptures, SCL and BM were not systematically recorded. We obtained 9,680 and
128 1,432 measurements of both SCL and BM from Golem Grad and Konjsko
129 populations, respectively.

130 During field work, we examined all dead (or dying) tortoises. In total, we found
131 315 dead tortoises on Golem Grad (223 males, 41 females and 51 immatures; this
132 proportion of dead males reflects the highly biased sex-ratio in this site; Bonnet et al.
133 2016) and 35 in Konjsko (20 males, 14 females and 1 immature). Most of them were
134 partly rotten or fully skeletonised (i.e. empty shells) and could not be used for
135 dissection. We retained only 13 very fresh individuals for dissection (12 from Golem
136 Grad and one from Konjsko; Table 1). Most of them had big cracks on the shell,
137 revealing that they fell from cliffs, or serious injuries on the cloaca suggesting they
138 had died from sexual harassment (Golubović et al., 2013, 2018). Animals in poor
139 condition (e.g. severely wounded) but still alive were kept in outdoor enclosures,

140 wounds cleaned, and provided with water and food until they recovered or not. One
141 recently wounded female could not be cured in the field (very deep hole in the back),
142 thus she was euthanized.

143 Prior to dissection, we recorded SCL and BM. Then we opened the shell ventrally
144 using a hacksaw to remove the plastron. The main organs of the tortoise were
145 separated (using scissors and pliers), carefully examined and weighed using
146 electronic scales. The mass of the shell was recorded to the nearest 0.1 g. Most
147 components could be actually separated and weighed with precision of 0.1 g: heart,
148 liver, lungs, stomach, intestine, gonads, growing follicles, eggs or digestive tract
149 content for example. Several parts of the body could not be easily dissected. For
150 example, we did not tease apart the skin, muscles and bones for the legs, head, neck
151 and tail; thus, we only recorded the total mass of the abovementioned elements.
152 Fluids and blood lost during dissection, along with parts which were not easily
153 identified or assigned, like mesenteric tissues and blood vessels for example, were
154 classified as miscellaneous material for conciseness.

155

156 *Analyses*

157 Body condition index (BCI) was calculated using residuals from a linear regression
158 (living and dissected individuals pooled, dead but not-dissected tortoises discarded)
159 of \ln -BM against \ln -SCL (Bonnet et al., 2001). This allowed us to evaluate to what
160 extent the BCI of freshly dissected tortoises was representative of the values
161 measured in the studied populations. The small proportion of dissected tortoises
162 (0.1%) limited their contribution in the calculation of the parameters of the
163 regression. BCI values were normally distributed (Shapiro-Wilk test, $p > 0.05$). Many
164 living individuals were represented more than once (recaptured over time),
165 therefore, individual identity was included as a random factor. Not-dissected dead
166 tortoises (e.g. partly rotten, empty shells) were not included in the body condition
167 analyses, but they were plotted on Figure 1 for comparison. Generalized linear
168 mixed models were built in R Version 1.2.5001 (R Core Team, 2019) using the

169 package nlme (Pinheiro et al., 2019). Competitive models were ranked using the
170 Akaike information criterion (AIC, Burnham and Anderson, 2002).

171 The masses of certain organs were highly correlated (e.g. left *versus* right legs).
172 Furthermore, for the purpose of the current study, considering morpho-functional
173 components instead of each separate element improved the conciseness of analyses.
174 Therefore, we assigned all dissected elements into 11 simple units (henceforth
175 designated as components, Table 2) that were used in most analyses. The
176 relationship between the total body mass and the respective mass of these
177 components was examined with a backward stepwise regression. We first verified
178 that the mass of these components, used as the dependent variables, was correlated
179 ($0.57 < r < 0.98$; all $p < 0.05$) with the body mass (the independent variable). Because
180 the sum of the components represented total body mass, this stepwise analysis
181 enabled us to identify potential major contributors of BCI.

182 Sex difference in body composition was tested using ANCOVA with the main
183 component as the dependent variable, sex as the factor and body mass as the
184 covariable. The strong correlation between the mass of each body component and
185 body mass facilitated this analysis. However, due to the small sample size of
186 dissected tortoises, we also used non-parametric Kruskal-Wallis ANOVA to
187 compare the body composition (e.g. shell, somatic and visceral organs mass relative
188 to the body mass, ratios were arcsine transformed prior to the analyses) of females
189 ($N = 4$) relative to males ($N = 9$).

190

191 **3. Results**

192 *Body condition: effect of sex, age and population*

193 The data obtained enabled us to encompass the range of variations of body mass as a
194 function of body size in both populations (Figure 1). Model selection revealed that
195 interaction of locality (Golem Grad Island or Konjsko village), state (living *vs.*
196 dissected) and sex (male, female or immature) best explained variation of BCI (Table
197 3). Tortoises from Konjsko exhibited higher body condition in general, regardless of

198 state and sex ($t = 9.88$, $p < 0.001$, Figure 1), whereas males exhibited lower body
199 condition than females in both localities ($t = -3.33$, $p < 0.001$).

200

201 *Body condition of living and dissected individuals*

202 Dissected tortoises exhibited lower body condition compared to living individuals (t
203 $= 4.0$, $p < 0.001$), but Figure 1 shows that the body mass relative to body size of the
204 dissected tortoises remained within the range of variation exhibited by living ones,
205 especially in Golem Grad where most dissected tortoises were found (Figure 1A).
206 But considering the Konjsko population, most dissected tortoises could be classified
207 as lean (Figure 1B).

208

209 *Body composition of dissected tortoises*

210 Body composition was highly variable among dissected individuals (Table 1). For
211 example, focusing on adult males, the relative mass of the shell ranged from 33.5% to
212 51.8%. This strong variability was also observed in other body components (see
213 Supplementary Material - Table I).

214 The final step of the backward regression (adjusted $R^2 = 0.999$, $F_{9, 3} = 4894.2$, $p <$
215 0.0001) retained nine components among eleven, that significantly contributed to
216 total body mass (Table 4). The masses of somatic organs (legs, head, neck and tail)
217 and shell mass were the most important contributors (standardized coefficients $>$
218 0.30). Miscellaneous material and digestive tract (net) were also substantial
219 contributors (standardized coefficients > 0.10). Finally, the masses of kidneys,
220 digestive tract content (i.e. partly digested material), and fat bodies, were retained in
221 the model, but the standardized coefficients were small (~ 0.05). The masses of the
222 heart and respiratory system provided almost null standardized coefficients (~ -0.08).
223 The masses of the liver and reproductive organs were not retained.

224 Regarding sexual dimorphism, ANCOVA and Kruskal-Wallis ANOVA provided
225 similar trends (although some non-parametric tests failed to reach statistical
226 significance). We found sex differences for the most important components: females
227 exhibited relatively heavier shells, while males showed higher relative somatic

228 organs mass – (Table 5; Figure 2). A closer inspection of the somatic organs showed
229 that males had relatively heavier limbs and heads (Table 5). They also had higher
230 relative values of some visceral organs, e.g. hearts and livers compared to females
231 (Table 5). We found no other significant sex differences.

232

233 **4. Discussion**

234 Dissections of free-ranging tortoises provided new insight on the significance of
235 body mass, and by extension of body condition in terrestrial chelonians. Several
236 results support previous studies (e.g. about sexual dimorphism), but others were
237 unexpected (e.g. regarding the relative contribution of digestive tract content *versus*
238 fat bodies).

239

240 *Dissected versus living tortoises*

241 On average, dissected individuals were in the lower part of the range of variation of
242 BCI exhibited by living tortoises (Figure 1). Dead tortoises may dehydrate rapidly in
243 the field, especially those with large cracks in the shell. Some tortoises may have lost
244 significant amounts of blood. Rapid loss of fluids may reduce body mass and thus
245 body condition. One female harassed by nine males exhibited low body condition
246 and may have died from exhaustion. On the other side of the spectrum, our sample
247 also included one large female with a high BCI.

248 Overall, our dissection data encompass most of the variation in adult body size,
249 but are not fully illustrative of the whole range of body condition observed in the
250 populations monitored (e.g. heaviest males and small individuals were lacking).
251 However, comparisons with other studies provide means to assess to what extent
252 our dissection results are representative of what is currently known in chelonians.

253

254 *Shell mass*

255 The protective role of the armour is crucial in Chelonians (Polly et al., 2016), but it
256 also imposes major morpho-functional and energetic constraints. The shell can
257 obstruct movements in dense vegetation and limits tortoise agility (Bonnet et al.,

258 2001; Golubović et al., 2014, 2015; Chiari et al., 2017). Abundant resources and time
259 are needed to build an effective shell; this shapes the growth trajectory during the
260 first decade of life in the Hermann's tortoise (Arsovski et al., 2018a, b). Likely, crucial
261 structural materials of the shell are not easily mobilised; we thus speculate that they
262 play no (or limited) role as body reserves.

263 The fresh mass of the shell represents the main component of a tortoise, averaging
264 41.5% of total body mass (39.6% when removing two extreme values in very lean
265 individuals). Dissections of captive tortoises indicate that the mean dry mass of the
266 shell relative to the dry mass of the carcass represents 31%, and 35% in adults (Table
267 18 in Kopsch, 2006). Despite differences in methodologies (fresh *vs.* dry masses,
268 relative to total body *vs.* carcass mass), previous and current studies show that the
269 mass of the shell is considerable in tortoises (suggested by Iverson 1984). This
270 proportion is approximately 30% (27% in adults) in terrapins (Miller and Birchard,
271 2005; Kopsch, 2006), and is markedly lower (17%) in adult sea turtles (Sakai et al.,
272 2000).

273 We observed very strong variations among individuals in the relative fresh mass
274 of the shell (34% to 52%). Dry masses measured in different species support our
275 results (e.g. 23% to 80%; Kopsch, 2006). The mass of the shell correlates with body
276 size, yet not perfectly ($r^2 = 0.81$, $N = 13$) due to variations in shell shape. Part of the
277 variation in total body mass (~19%) is caused by interindividual variations in
278 relative shell mass. Thus, BCI includes an unknown albeit substantial shell-
279 component independent of body reserves.

280

281 *Body reserves*

282 Fat bodies were virtually absent in all dissected individuals, representing only 0.0%
283 to 0.5% of the total body mass. Kopsch (2006) did not provide information regarding
284 fat bodies in the dissections of 92 captive individuals (3 *Testudo*, 2 terrapins, and
285 *Geochelone pardalis*); suggesting that adipose tissues were negligible. Sporadic cases
286 of obesity have been reported in overfed captive tortoises, with pads protruding
287 from the openings of the shell (Burchfield et al., 1980; Lamberski, 2013). But this

288 situation may not occur in natural conditions: we never found an obese individual in
289 our large datasets (> 5,000 individuals) collected in different tortoise species (Bonnet
290 et al., 2001; El Mouden et al., 2006; Djordjević et al., 2011; Lecq et al., 2014).
291 Noticeably, in the current study, dissected individuals exhibited average to low
292 body condition (except one very large female). Thus, we cannot ascertain that fat
293 bodies are always negligible, especially in heaviest individuals. It would be
294 informative to assess if fat bodies develop above a BCI threshold (this was not the
295 case in the high-BCI dissected female, 0.3% fat). Abundant fat deposits, however,
296 have been observed in sea turtles (Kwan, 1994), reaching 12% of the total body mass
297 in female loggerhead turtles (calculated from Table 1 in Sakai et al., 2000). Lipidic
298 reserves may well be more developed in aquatic than in terrestrial chelonians.

299 The liver is an important source of endogenous reserves (e.g. lipids and proteins)
300 mobilized during reproduction (Telford, 1970; Bonnet et al., 1994; Heck et al., 1997;
301 Finkler, 2013). This organ also stores reserves beyond reproduction (Collins and
302 Anderson, 1995). Dissections showed that the liver represents an important
303 proportion of body mass in Chelonians, 2.7% to 7.2% in the current study, 2.6% to
304 3.5% in female sea turtles (Sakai et al., 2001) and 1.5% to 5.0% in tortoises and
305 terrapins (Table 32 in Kopsch, 2006).

306 Fat bodies, the most “classical” proxy for BCI, did not contribute significantly to
307 variation of body mass in tortoises. Somatic organs (e.g. locomotor muscles) and the
308 digestive tract were better contributors. Tortoises may first allocate resources in their
309 locomotor and digestive structures, and build up fat bodies only later. Future
310 investigations focusing on the relationships between body condition and dynamic
311 aspects of mass variation of the main components are needed.

312

313 *Sexual dimorphism in body plan*

314 In our study, the relative mass of the shell was greater in females (38.7% – 52.3% *vs.*
315 33.5% – 51.8% in males; Supplementary Material, Table I). This supports the notion
316 of a more developed shell in females to maximize protective functions and to
317 enhance abdominal volume to hold the clutch, compared to a relatively lighter shell

318 with larger openings to favor mobility in males (Bonnet et al., 2001; Djordjević et al.,
319 2011). The relative mass of organs involved in locomotor performance (e. g. limbs,
320 heart) was greater in males compared to females (21.5% – 30.0% *versus* 15.8% – 24.3%
321 of the total body mass; Supplementary Material, Table I). These result fit well with
322 previous studies on sexual dimorphism of body plan in chelonians (Bonnet et al.,
323 2010), and more generally regarding sexual dimorphism of body composition in
324 snakes and fishes (Bonnet et al., 1998; Casselman and Schulte-Hostedde, 2004).

325

326 *Exogenous components inside the body*

327 In all dissected individuals, dissections revealed substantial amounts (4.4% – 10.2%
328 of the total body mass in adults) of exogenous and partly digested materials in the
329 digestive tract. Incidentally, the highest proportion (14.5%) was observed in the
330 immature male; perhaps small and rapidly growing individuals need to feed a lot.
331 Regardless, partly processed food contributed to total body mass more than fat
332 bodies.

333 The resources invested into large follicles and ovulated eggs cannot be considered
334 as endogenous reserves (Bonnet et al. 1994). One female carried abundant and well-
335 developed follicles and eggs (Supplementary Material), but in the others, the ovaries
336 and oviducts were almost empty, only small follicles being visible. This considerable
337 variation (0.1% – 10.0% of the total body mass) explains the significant contribution
338 of the reproductive organs + follicles and eggs in total body mass variations.

339

340 *Body condition versus body composition*

341 Body condition integrates the mass of endogenous body reserves (fat and protein
342 stores) along with exogenous material. Fluctuations of the relative mass of the shell
343 cannot be factored out. Further, hydration status influences body mass (Packard and
344 Packard, 2001), as suggested by the importance of the fluids lost during dissections.
345 Thus, the meaning of body condition should be considered with caution and
346 certainly not reduced to lipidic stores. Yet, BCI is a useful index to assess health
347 status in tortoises; for example, it strongly correlates with the mass of crucial

348 locomotor structures. Additionally, in females it may well be a good indicator of
349 reproductive status. The mass of the reproductive items of the dissected female with
350 well-developed eggs and follicles was 5 to 100 times greater than in non-
351 reproductive females (Supplementary Material - Table I).

352

353 *Consequences for ecological studies*

354 The two populations we studied differ considerably in terms of body condition; this
355 contrast requires explanation(s). Only one individual from Konjsko was dissected,
356 precluding direct comparisons of body composition between sites however. In
357 Golem Grad fat bodies were negligible, but this might not be true for the heaviest
358 Konjsko tortoises. Yet, using BCI as a mere indicator of fat stores may lead to invalid
359 conclusions. For example, on Golem Grad, tortoises with a high BCI might be
360 incorrectly considered as fat compared to those with a low BCI - a classical shortcut
361 in ecological studies. Instead, our results suggest that somatic organs were more
362 important. This information may help to address specific issues. For instance, we
363 may hypothesize that Golem Grad tortoises are locally adapted to extremely uneven
364 topography where lightness and agility are advantageous (Golubović et al., 2017).
365 Alternatively, does low food availability explain why Golem Grad tortoises are lean
366 compared to Konjsko? The ecological and evolutionary processes that underpin
367 these questions differ considerably. Well-informed BCI values would be then crucial.

368 In conclusion, field dissections provided support to previous studies on sexual
369 body shape dimorphism. More importantly, they enabled us to broaden the meaning
370 of body condition in free-ranging tortoises, notably to shift away from a fat store
371 focus toward a more complex vision that includes various body components. This
372 might be useful for future studies of sexual dimorphism, feeding ecology,
373 reproductive investment, demography, and other life-history traits.

374

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382

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Table 1. Summary data of dissected tortoises from Golem Grad Island (GG) and Konjsko village (KV). M (I) – immature male, M – adult male, F – adult female. SCL stands for straight carapace length (mm), BM1 and BM2 respectively for body mass measured when the animal was found (dead or alive, see text) or just before dissection. BCI means Body Condition Index (mass scaled by size, residuals). The most important components of total body mass are provided (shell, soma and viscera). Soma includes front and hind legs, head, neck and tail; Viscera includes liver, heart, thymus, spleen, respiratory organs, kidneys, reproductive organs, digestive system (stomach + intestine + food + faeces), fat bodies, miscellaneous material (blood. etc.).

Sex, ID and location	Biometry			Mass at dissection (g)			
	SCL	BM1	BCI	BM2	Shell	Soma	Viscera
M (I) 994 (GG)	95.9	211.0	0.08945	203.0	81.0 (39.9)	56.7 (27.9)	65.3 (32.2)
M 843 (GG)	182.2	1199.0	0.00065	1016.5	397.7 (39.1)	387.0 (38.1)	231.8 (22.8)
M 234 (GG)	188.0	1113.0	-0.16300	1062.0	393.9 (37.1)	418.3 (39.4)	249.8 (23.5)
M 722 (GG)	148.0	426.0	-0.44304	423.0	219.0 (51.8)	133.3 (31.5)	70.7 (16.7)
M 303 (GG)	139.2	556.0	-0.00222	555.0	224.0 (40.4)	179.2 (32.3)	151.8 (27.3)
M 1296 (GG)	187.0	1151.0	-0.11485	1078.0	473.0 (43.9)	360.5 (33.4)	244.5 (22.7)
M 1206 (GG)	174.0	968.0	-0.08291	964.0	323.0 (33.5)	381.3 (39.6)	259.7 (26.9)
M 924 (GG)	186.0	1286.0	0.01132	1220.0	450.0 (36.9)	453.6 (37.2)	316.4 (25.9)
M 338 (GG)	178.0	1076.0	-0.04183	1063.6	395.0 (37.1)	361.6 (34.0)	306.4 (28.9)
F 1776 (GG)	152.0	672.0	-0.06313	651.0	278.0 (42.7)	187.7 (28.8)	185.3 (28.5)
F 1058 (GG)	178.0	911.0	-0.20830	914.0	424.0 (46.4)	276.6 (30.3)	213.4 (23.3)
F 1712 (GG)	163.0	766.0	-0.13108	771.0	403.0 (52.3)	161.5 (20.9)	206.5 (26.8)
F NEW (KV)	225.0	2348.0	0.07152	2348.0	908.0 (38.7)	629.0 (26.8)	811.0 (34.5)

Table 2. List of the main components used to describe the body composition of the dissected tortoises. They were implemented in the stepwise backward regression analysis. Element pooled explain which organs or elements were pooled in certain components.

Component (g)	Elements pooled
Shell	-
Somatic organs	See Supplementary Material – Table I
Liver	-
Heart	-
Respiratory system	-
Kidneys	-
Reproductive organs	Both gonads, epididymis, oviducts plus follicles and ovulated eggs
Digestive tract	Gullet, stomach and intestine (net)
Fat bodies	-
Digestive tract content	Stomach and intestine content
Miscellaneous material	Blood, lymph, several mesenteric elements and blood vessels

Table 3. Model ranking of generalized linear mixed models of Hermann’s tortoise body condition variation conditional to univariate, additive (+) and interactive (*) effects of sex and state (immature, male or female) and locality (Golem Grad Island and Konjsko village). One model built to evaluate whether dissected tortoises’ body condition differed from that of living tortoises (dissected *vs.* alive) had a low AIC (likely due to small sample size of dissected tortoises). Models were ranked according to their respective Akaike Information Criteria (AIC) values, Δ AIC and AIC weights.

Model	AIC	ΔAIC	AIC weight
Dissected <i>vs.</i> alive	-28.734,8	479.6	0.0
Sex and state	-28.905,9	308.5	0.0
Locality	-29.204,2	10.2	0.0
Sex + Locality	-29.214,2	0.2	0.5
Sex * Locality	-29.214,4	0.0	0.5

Table 4. Final selection from the stepwise backward regression analysis (following 7 steps). Nine components among 11 (see Table 2) were retained. Standardized coefficient and coefficient are expressed \pm SE; t-test t with degree of freedom in brackets.

Component	Standardized coefficient	Coefficient	t (6)	p
Somatic organs	0.448 \pm 0.027	1.458 \pm 0.087	16.781	<0.001
Shell	0.314 \pm 0.020	0.836 \pm 0.052	15.969	<0.001
Miscellaneous material	0.208 \pm 0.014	1.975 \pm 0.129	15.223	<0.001
Digestive tract	0.124 \pm 0.014	2.609 \pm 0.282	9.280	<0.003
Kidneys	0.060 \pm 0.014	9.362 \pm 2.196	4.265	0.025
Digestive tract content	0.058 \pm 0.013	1.014 \pm 0.219	4.631	0.019
Fat bodies	0.047 \pm 0.013	11.218 \pm 3.233	3.470	0.040
Heart	-0.083 \pm 0.018	-15.591 \pm 3.360	-4.641	0.018
Respiratory system	-0.076 \pm 0.018	-5.499 \pm 1.315	-4.183	0.025

Table 5. Results from GLM (ANCOVA, columns 2 to 4) and Kruskal-Wallis ANOVA (columns 6 and 7) to compare the relative mass of different components or organs between the sexes (4 females and 9 males). SD (column 5) indicates the direction of sexual dimorphism. Components where only non-significant results were found are not reported.

Component	SC	F	P	SD	H	P
Shell	9089.3	7.466	0.021	F	2.381	0.122
Somatic organs	21476.3	12.192	0.006	M	6.095	0.013
Legs	9732.0	12.472	0.005	M		
Head	424.8	8.385	0.016	M		
Heart	29.3	12.510	0.005	M		
Liver	1508.9	10.633	0.009	M	5.357	0.020

Figure captions:

Figure 1. Relationship between body mass (g) and body size (straight carapace length, mm) of live tortoises from Golem Grad Island (Top panel A) and Konjsko village (Lower panel B). Dissected (black circles), and not dissected but dead (grey crosses) tortoises are displayed on both panels for population comparison (scales identical). One large dissected female was in very good body condition and one male was particularly lean (grey arrows). This large female was found in Konjsko, all the other dissected tortoises were found in Golem Grad.

Figure 2. Comparison of the body composition of male and female Hermann's tortoises. Mean values (\pm SD) provide the proportions (% relative to body mass) of the main body components: shell, somatic organs, visceral organs, fat bodies and faeces (see table 2 and text for details and statistics).

Figure 1.

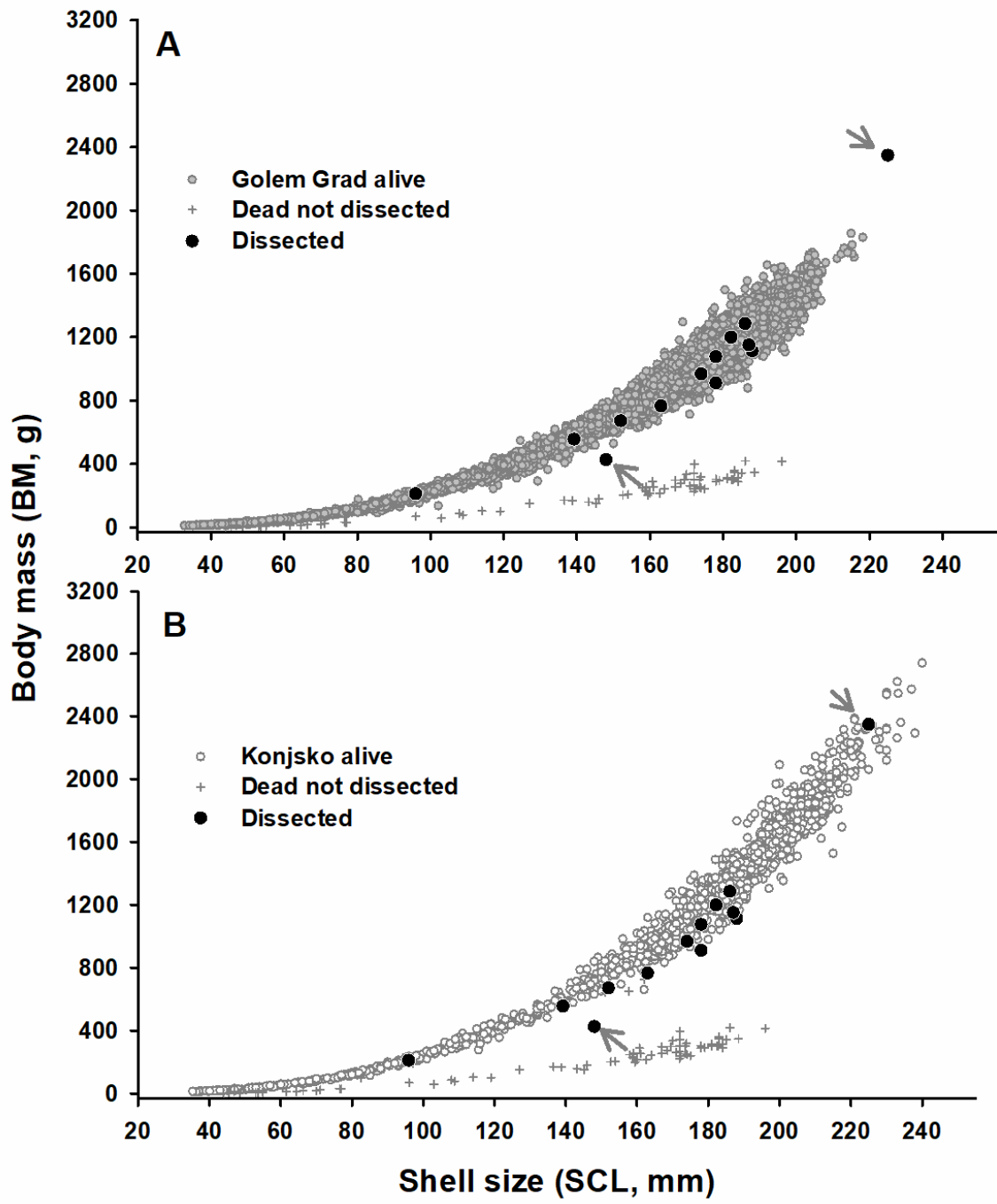


Figure 2.

